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Toxicity of Aldicarb and Fonofos to the Early-Life-Stage of the Fathead Minnow

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Toxicity of Aldicarb and Fonofos to the Early-Life-Stage of the Fathead Minnow

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Abstract. Flow-through early-life-stage (ELS) toxicity tests were conducted with the pesticides aldicarb (Temik®) and fonofos (Dyfonate®) to determine their effect on the survival and growth of fathead minnows. Concentrations of 78 μg/L of aldicarb and 16 μg/L of fonofos did not affect survival and growth. However, 156 μg/L of aldicarb and 33 μg/L of fonofos were lethal to larval-juvenile exposed for 30 days post-hatch. The maximum acceptable toxicant concentration (MATC) of aldicarb and fonofos for the fathead minnow is estimated to be between 78–156 μg/L and 16–33 μg/L, respectively. The corresponding chronic values (geometric mean of MATC values) would be 110 μg/L and 23 μg/L. Acute toxicity tests gave 96-hr LC50 values of 1370 μg aldicarb/L and 1090 μg fonofos/L. The acute-chronic ratio (96-hr LC50/chronic value) is 12 for aldicarb and 47 for fonofos.

This study is part of a research program to develop information on the biological effects of some newer pesticides to non-target aquatic animals. Kelso *et al.* (1978) have estimated the pollution potential of 82 major synthetic organic pesticides. A pesticide priority rating was developed on the basis of production, effects, and fate. In the pollution potential rank order of the 82 pesticides, fonofos ranked 23rd and aldicarb ranked 27th. The 1974 production of these pesticides was estimated to be 3 million pounds for fonofos and 5 million pounds for aldicarb. Both compounds had a high acute toxicity rating.

McKim (1977) and Macek and Sleight (1977) reviewed the literature on chronic toxicity tests with fish and indicated that in the majority of cases the embryo and larval-juvenile stages were the most sensitive life-cycle stages. They concluded that an early-life-stage toxicity test was a good short-term method to estimate maximum acceptable toxicant concentrations (MATC) as measured by life-cycle tests. In the development of water quality guidelines (Federal Register 1980), chronic toxicity is based on life-cycle, partial life-cycle, or early-life-stage toxicity tests. The chronic value of a pollutant is the geometric mean of the lower and upper chronic limits from a chronic test.

The purpose of this study was to investigate the effects of these two pesticides on the fathead minnow (*Pimephales promelas*). Chronic toxicity was estimated on the basis of early-life-stage (ELS) toxicity tests. These exposures were initiated with eggs less than 24-hr old and continued through 30 days post-hatch. The effects on embryo survival and on survival and growth of the larvae were measured and used to estimate chronic toxicity values. In addition, acute toxicity studies were made to determine 96-hr LC50 values to derive acute-chronic ratios.

Methods and Experimental Conditions

Exposure System: In both ELS toxicity tests, a proportional diluter, with a dilution factor of 0.5, was used to deliver five pesticide concentrations and control to duplicate exposure chambers. These stratified randomly-arranged all-glass chambers, 60 × 15 × 15 cm, were calibrated to contain a water volume of 8 L and to receive a flow of 60 L per day. Embryos were incubated in glass cylinder cups with nylon screen glued to the bottom to provide water exchange with the chambers. The cylinders were vertically oscillated by attaching them to a rocker-arm apparatus driven by a 2-rpm motor. Automatically controlled fluorescent lamps provided a 16-hr light cycle. The dilution water was a constant quality mixture of spring water and carbon-filtered, demineralized Cincinnati tap water that was mixed in a covered concrete reservoir and had a mean retention time of 14 days.

Biological System: The ELS toxicity tests were initiated with 50 embryos per cup in each duplicate exposure chamber. These embryos from seven substrates from an indoor culture unit were less than 1-day old. At complete hatch, larvae were transferred from the cup to the exposure chamber and exposed for 30 days. Forty larvae per chamber were used in the aldicarb exposure, and 30 larvae per chamber were used in the fonofos exposure. These larvae were fed newly hatched brine shrimp three to five times per weekday and two to three times per day on weekends. Exposure chambers were cleaned at least once a day. After the exposure, the fish were blotted dry and weighed.

Physical and Chemical Conditions: Routine analyses of water from the exposure chambers were made weekly using procedures described by the American Public Health Association *et al.* (1975). The aldicarb exposure had mean and standard deviation measurements for dissolved oxygen, hardness, alkalinity, and pH of 8.2 ± 0.1 mg/L, 198 ± 7 mg/L (as $CaCO_3$), 179 ± 10 mg/L (as $CaCO_3$), and 7.9 ± 0.1, respectively. The fonofos values were 7.5 ± 0.5 mg/L, 199 ± 6 mg/L (as $CaCO_3$), 170 ± 6 mg/L (as $CaCO_3$) and 7.5 ± 0.1, respectively. The mean temperature was 23 ± 1°C in the aldicarb exposure and 24 ± 1°C in the fonofos exposure.

Technical-grade (99% purity) aldicarb [2-methyl-2-(methylthio) propionaldehyde *O*-(methylcarbamoyl)oxime] was used daily to prepare an aqueous solution that was introduced from a Mariotte bottle and a siphon-activated metering device. The nominal toxicant solution delivery was 18.0 ml to give a high concentration of 320 μg/L. The mean daily measured delivery was 18.4 ± 0.7 ml. Each concentration of aldicarb was measured once a week from grab samples of the duplicate exposure chambers. Analyses were made on duplicate sample concentrations and spiked control water in each sample set. Spiked samples were analyzed with the samples against known standards to give recovery rates. Samples were extracted with methylene chloride and analyzed by gas chromatography (GC) using a method based on work done by Carey and Helrick (1970). Aldicarb and its metabolites were oxidized to the sulfone, chromatographed as the sulfone oxime by pre-column degradation, and detected with an electrolytic conductivity detector in the sulfur specific mode. Recoveries of spiked control water were consistently concentration-dependent and ranged from an average of 63% ± 10% in the low concentration to an average of 91% ± 6% in the high concentration. Eighty-five determinations were made on samples, spikes, replicates, and controls. The results were corrected for recovery and are given in Table 1.

An acetone solution of technical-grade fonofos [*O*-ethyl-*S*-phenyl ethyl phosphonodithioate] was added daily to deionized water and introduced from a Mariotte bottle and a siphon-activated metering device. The nominal toxicant high concentration was 80 μg/L of fonofos and 4.0 mg/L of acetone. In addition, a second metering system was used to give an acetone control with a nominal concentration of 4.0 mg/L. Both sets of the duplicate exposure tanks were sampled once a week for pesticide analysis. Dilution water was sampled along with each set and spiked with pure standard to concentrations equivalent to each test concentration. The spiked samples were analyzed with the samples against known standards for recovery rates. Samples were extracted with petroleum ether, concentrated, and measured by GC using an electron capture detector. During the study, 132 separate extractions were made and analyzed. Overall, spiked recoveries were 89 ± 8.2%. For duplicate determinations, average deviation from the mean value was 3.6 ± 2.5%. Sample values were corrected for recovery and are given in Table 2.

Acute Toxicity: The ELS diluter system with similar dilution water was used for a flow-through aldicarb acute exposure using methods recommended by U.S. EPA (1975). The high nominal concentration was 3,200 μg/L, with a 0.5 dilution factor. Ten juveniles (mean wt. 0.92 g) were used in each duplicate exposure chamber. Fish were exposed at 22 ± 1°C for four days with samples for aldicarb analysis being taken initially and on the fourth day. The deviation from the mean of these two sets of analysis was <5%, so the mean values were used in the LC50 calculation. At 96-hr, the exposure to the pesticide dosing was stopped, and the fish were exposed to dilution water only for an additional seven days.

Because of low solubility (12–15 mg/L) of fonofos, a 96-hr static test with measured concentrations, instead of flow-through tests, was conducted according to the methods recommended by U.S. EPA (1975). Duplicate series of ten juveniles (mean wt. 0.34 g) per 10 L wee used with a high concentration of 5.6 mg/L and a dilution factor of 0.56. Samples for pesticide analysis were taken initially and after 100% mortality or on the fourth day. The mean of the two measurements was used in the LC50 calculation. The measured concentrations were somewhat less than the nominal concentrations; these measured concentrations did not decrease with time. The exposure was conducted at 22 ± 1°C. The dilution water had an alkalinity and hardness of 168 and 192 mg/L (as $CaCO_3$), respectively. During the test, the pH decreased from 8.2 to 7.9. Dissolved oxygen also decreased, but did not go below 60% saturation.

Statistical Analysis: Embryo survival (hatchability), larva survival, and the weight of individual fish alive at the termination of the test were subjected to one-way analysis of variance. When significant ($P = 0.05$), the above values were also subjected to Dunnett's one-sided comparison of concentration to control means ($P = 0.05$) (Steel and Torrie 1960) for the aldicarb exposure, and to acetone control means for the fonofos exposure. Percentage survival data were transformed using an arcsin transformation, treating the test chambers as replicates. The weights of individual fish in both duplicate exposure chambers were combined and used as one group of observations. The LC50 values and 95% confidence limits were determined by probit analysis (Finney 1971).

Results

Survival: Hatchability of embryos in the aldicarb and fonofos exposures was excellent, where survival varied from 92% to 98% (Tables 1 and 2). The statistical analysis indicated that there was no significant difference between embryo survival in pesticide concentrations and control. However, 30-day post-hatch survival of these larvae at the two high concentrations of both pesticides (Tables 1 and 2) was significantly reduced. Concentrations of 156 μg/L of aldicarb and 33 μg/L of fonofos were lethal to the fathead minnow.

Survival of fish in the acute exposure was as given in Table 3. The 96-hr LC50 of aldicarb was 1,480 (1,170–1,890) μg/L based on nominal concentrations, and 1,370 (1,169–1,730) μg/L based on measured concentrations. Because several fish in different concentrations of aldicarb had lost equilib-

Table 1. Survival and growth of early-life-stages of fathead minnows exposed to aldicarb

Measured concentration (μg/L) (mean ± S.D.)		Embryo survival (%)	Larva-juvenile survival (%) at 30 days	Juvenile weight (mg) (mean ± S.D.) at 30 days
340 ± 7.8	A	98	25	84.2 ± 41.0*
	B	96	15*	
156 ± 13.0	A	96	50	124.0 ± 32.0
	B	98	33*	
78 ± 5.9	A	96	93	141.3 ± 36.8
	B	96	90	
38 ± 3.3	A	98	90	140.2 ± 36.8
	B	94	90	
20 ± 1.4	A	96	93	139.0 ± 34.5
	B	98	85	
Control	A	94	93	149.7 ± 34.7
	B	96	90	

* Significantly different (P = 0.05) from control

Table 2. Survival and growth of early-life-stages of fathead minnows exposed to fonofos

Measured concentration (μg/L) (mean ± S.D.)		Embryo survival (%)	Larva-juvenile survival (%) at 30 days	Juvenile weight (mg) (mean ± S.D.) at 30 days
66.0 ± 8.6	A	94	43	43.6 ± 15.8*
	B	96	23*	
33.0 ± 4.9	A	94	77	117.7 ± 46.7
	B	92	57*	
16.0 ± 2.8	A	96	97	149.9 ± 36.0
	B	96	87	
9.2 ± 2.1	A	96	93	178.9 ± 55.4
	B	98	100	
4.9 ± 1.4	A	94	97	199.8 ± 51.7
	B	96	87	
Control (acetone)	A	94	87	167.5 ± 49.0
	B	94	97	

* Significantly different (P = 0.05) from control (acetone)

rium by 96-hr exposure, the test was continued for an additional seven days without the addition of aldicarb. During four days post-exposure, there was some delayed mortality, but all surviving fish had recovered their equilibrium. There was no additional mortality during the last three days of clean water exposure. This LC50 value based on measured concentrations for the four day aldicarb exposure was 1,200 (980–1,500) μg/L. The 96-hr LC50 value for fonofos was 1,360 (1,130–1,630) μg/L, based on nominal concentrations, and 1,090 (930–1,260) μg/L based on measured concentrations.

Growth: The mean weight of the surviving fish exposed to the lethal concentration of 340 μg/L of aldicarb was 56% of the weight of the control fish (Table 1). The weight of the surviving fish at the lethal concentration of 156 μg/L was 17% lower than the weight of control fish, but was not statistically significant. At sublethal concentrations, the weights were not significantly less than the weight of the control fish. The mean weight of the surviving fish exposed to the lethal concentration of 66 μg/L of fonofos was only 26% of the weight of control fish and was significantly less than the weight of the fish in the acetone control. Growth of surviving fish at the lethal concentration of 33 μg/L and at sublethal concentrations was not significantly less than the acetone control fish.

Discussion

The results of the ELS toxicity test show that the MATC of aldicarb for the fathead minnow is between 78 and 156 μg/L. At concentrations of 78 μg/L and less, there was no adverse effect on embryo survival or on larval-juvenile survival and growth. Survival of larvae-juveniles after 30 days exposure to 156 μg/L was significantly less than survival of control fish. Survival was less than 50% at this concentration.

There are no available studies on the chronic toxicity of aldicarb to other fish species. However, there is a life-cycle chronic study on the toxicity of another carbamate pesticide to the fathead minnow. Carlson (1971) reported an MATC for carbaryl that was between 210 and 680 μg/L. The high concentration had an adverse effect on egg production; however, this effect was not more sensitive than the effect on survival of first generation fish. Assuming a similar mode of action for aldicarb and carbaryl, these data suggest that the MATC for aldicarb using the ELS exposure is a good estimate of chronic toxicity.

The results of the fonofos exposure indicate that the MATC is between 16 and 33 μg/L. Survival and growth of fish exposed to 16 μg/L and less were not significantly different from control fish. Survival of fish exposed to 33 μg/L was only 67%, and this survival was significantly less than control fish. McKim (1977) reviewed the MATC values of life-cycle chronic tests and MATC values estimated from the embryo-larval and early-juvenile exposures conducted as part of the life-cycle tests. Three organophosphorus pesticides were evaluated in this review. For both diazinon and malathion, MATC

Table 3. Percentage survival of fathead minnows acutely exposed to aldicarb and fonofos

Concentration of aldicarb (μg/L)		Percentage survival			Concentration of fonofos (μg/L)		Percentage survival		
Nominal	*Measured*	*24 hr*	*48 hr*	*96 hr*	*Nominal*	*Measured*	*24 hr*	*48 hr*	*96 hr*
3,200	2,900	55	45	5	5,600	3,100	0	0	0
1,600	1,400	90	90	55	3,200	2,000	55	15	0
800	830	95	95	85	1,800	1,400	100	70	35
400	360	100	100	95	1,000	850	100	100	75
200	220	100	100	100	560	490	100	100	95
Control	Control	100	100	100	Control	Control	100	100	100

values from life-cycle tests were the same as obtained from the embryo-larval test. The MATC value for the life-cycle test with Guthion® was established on the basis of fecundity and was slightly lower than the MATC value estimated from the embryo-larval and early-juvenile exposure. The effect of all three pesticides was considered to be mediated through inhibition of acetylcholinesterase (AChE) activity, so it may be inferred that the MATC for fonofos probably is a good estimate of life-cycle chronic toxicity.

There is a wide range in the acute sensitivity of various fish species to organophosphorus pesticides. Pickering *et al.* (1962) reported that 96-hr LC50 values of 13 organophosphate insecticides for four species of fish ranged from four times for Chlorthion® to over 900 times for Delnav.® Mount and Stephan (1967) reported a MATC of 200–580 μg/L of malathion for the fathead minnow. This concentration is greater than the 96-hr (LC50 value of 110 μg/L (Eaton 1970) for the bluegill. The range in species sensitivity to aldicarb and fonofos is not known. Mount and Stephan (1967) proposed the use of experimentally derived application factors to estimate safe concentrations of a toxicant to other fish species. This application factor, MATC/96-hr LC50, was multiplied by the LC50 value of another species to estimate safe chronic concentrations for that species. Since fonofos is also a known AChE inhibitor, we feel our MATC value should reasonably predict the toxicity of this compound in a life-cycle chronic study.

Our ELS tests indicate a MATC for aldicarb between 78–156 μg/L, with a chronic value (geometric mean) of 110 μg/L. Using the 96-hr LC50 value of 1,370 μg/L and the chronic value of 110 μg/L, the acute-chronic ratio for aldicarb is 12. The estimated MATC for fonofos is between 16–33 μg/L, with a chronic value of 23 μg/L. Using the 96-hr LC50 value of 1,090 μg/L and the chronic value of 23 μg/L, the acute-chronic ratio for fonofos is 47. These acute-chronic ratios are proposed as application factors to be used for estimating safe concentrations of these two insecticides to other fish species.

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